The Impact of Technology on Assurance

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30 June, 2004

Overview

- Technology Applied to Reduce Risk
- Limitations and Constraints
- External Technology and Impact on Assurance
- Applying New Technologies – A New Tool Set
- Case Studies

Technology

tech·nol·ogy \ n: [Gk *tecnologia* systematic treatment of an art] **1**: technical language, **2** a: applied science **b**: a technical method of achieving a practical purpose **3**: the totality of the means employed to provide objects necessary for human sustenance and comfort

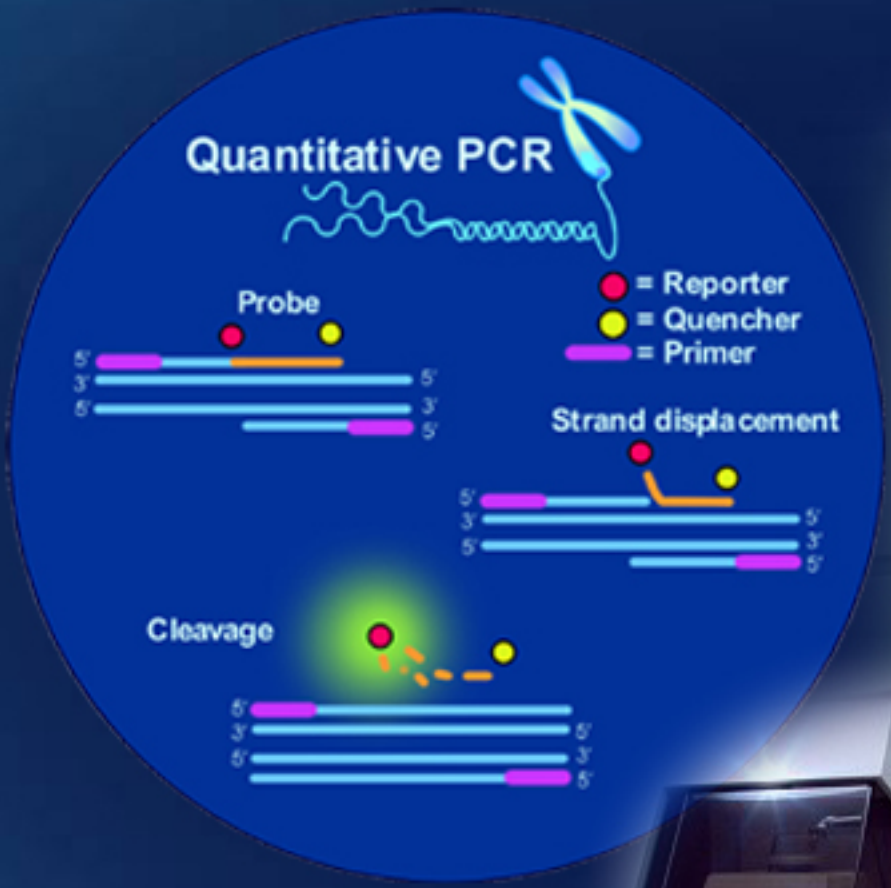
Webster's Seventh New Collegiate Dictionary,
G.& C. Merriam Company, Publishers, 1972

Thermus aquaticus

The large spring above, near Great Fountain Geyser, was the source of the culture of *Thermus aquaticus* that is used to make Taq polymerase, a key constituent of the polymerase chain reaction.

Thomas D. Brock, *Biotechnology in Yellowstone,*
1994 Yellowstone Association for Natural Science, History & Education, Inc. Yellowstone National Park, Wyoming 82190.

Quantitative PCR Services



Application of Best-Available Methods to Minimize Risk of Contamination

- Cell Substrates: Master Virus Bank
  Master Cell Bank
  Working Cell Bank
- Process: Biological Components
  Process Reagents
  Excipients
  Agents Introduced During Processing
- Target: Specific Agents of Concern, “Suspects” and Unknown Agents

Technologies Applied (ICH Q5A)

Assay System	Primary Intent	Basis/Technology
In Vitro Virus Assays	Broad Viral Screen	In Vitro Amplification/ Virus isolation and ID
In Vivo Virus Assays	Broad Viral Screen	In Vivo Replication/ Virus isolation and ID
Antibody Production Tests (MAP, HAP etc.)	Detection of Known "Suspects"	In Vivo Ab Formation/ Virus isolation and ID
TEM	Detection of "Suspects" and Unknown Viruses	Structural ID / Staining, Cytopathology
Biochemical (RT Assays)	Broad Detection Of Retroviruses	RV RT / Retroviral gene function, detection methodology
PCR	Specific Virus Detection	Specific sequences / Sequence Data

Technologies Applied (ICH Q5A)

Assay System	Assay Limitation	Practical Limitations
In Vitro Virus Assays	Agents Failing to Replicate	Toxicity, Artifacts
In Vivo Virus Assays	Agents Failing to Replicate	Artifacts, Subclinical symptoms,
Antibody Production Tests	Specific Viral Antigens, Virus-free animals	Serology data, antigen cross-reactivity
TEM	Qualitative Assay	Sample Quality, Complex Equipment
Biochemical (RT Assays)	Optimal Activity Only Under Preferred Reaction Conditions	Interpretation of Background in some Samples
PCR	No Indication of Infectivity	Specific Sequences Required

Integration and Biodistribution Studies

Integration and Biodistribution Studies are required for most DNA vaccines and gene therapy products.

Sensitive and Robust Assays

GLP Compliance

Increasing Efficiency in Throughput and Analysis

Application of Technology

- Use of Well Characterized Cell Lines (Sources)
- Master Cell Banks Produced from Certified Cell Seeds
- Redundant and Overlapping Testing Programs at Various Stages of Production

And Where Applicable,

- Use of Viral Inactivation and Removal Processes

Reduction / Limitation of Risk Through Improved Technology

- Improvements in Biological Reagent Quality
  Driven by:
  - Innovative Products Sans Processes for Agent Removal or Inactivation
  - Heightened Safety Concerns (BSE)
  - Improved Analytical Methods
  - Improved Testing Methods for Reagents
    - M. Barille (CBER) – Mycoplasma

Reduction / Limitation of Risk Through Improved Technology

- Clean in Place / Steam in Place
- Environmental and Facility Controls
- Environmental Monitoring Programs and Equipment
- Closed Reactor Systems, Automation and Disposables

* Challenges Remain for Processes with Limited Processing

“ As of February 12, 2001…We know the human genome consists of…

26,588 genes

0.9% Run our Immune System
2.9 % Prevent Tumors
3.3% Allow Cells to communicate with one another
5.0% Build Cells
10.2 % Make enzymes for Chemical Reactions
13.5% Run the Cell Nucleus

We don’t know what the other 41.7 % do…but the end count is
Likely around 30,000 genes…
You can put them all on a gene chip…
The size of a penny.”

Enriquez, Juan, **As the Future Catches You**, How Genomics and Other Forces are Changing Your Life, Work, Health and Wealth, 2001, Crown Business, New York, NY

External Technology Impacting Assurance

- Advances in Microarray Technology, Real-Time Data Capture and High Throughput Sensor Technology
- Genomics – Availability of Sequence Data Provides Understanding of Agents, Gene Expression and Ultimately, “Gene Signatures”
- Advanced Analytical Tools such as “Artificial Neural Nets”, “Evolutionary Computation” and “Valuated State Space” are Employed to Evaluate Sequence and Expression Data

Cytomegalovirus (CMV)

Hepatitis A Virus (HAV)*

Epstein-Barr Virus (EBV)

Hepatitis B Virus (HCV)

Human Immunodeficiency Virus Type 1 (HIV-1)*

Hepatitis C Virus (HCV)*

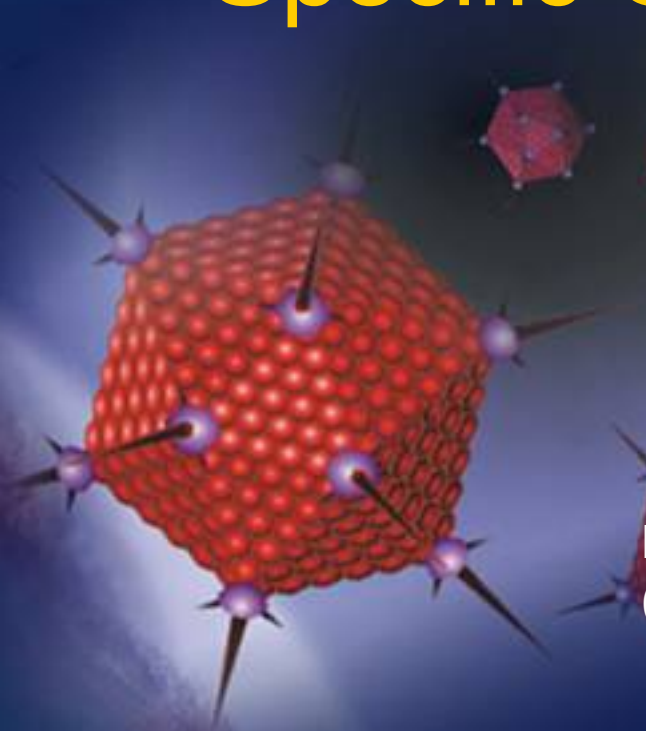
Human Immunodeficiency Virus Type 2 (HIV-2)*

Adeno-Associated Virus (AAV)

Parvovirus B19 (Parvo-B19)

Minute Virus of Mice (MVM)

Specific Genomic Assays Exist



Human Herpes Virus 6

Mycoplasma

Human T-Cell Leukemia Virus Type 1 (HTLV-1)*

Human T-Cell Leukemia Virus Type 2 (HTLV-2)*

Human Herpes Virus 7 (HHV7)

Human Herpes Virus 8 (HHV8)

*RNA Viruses

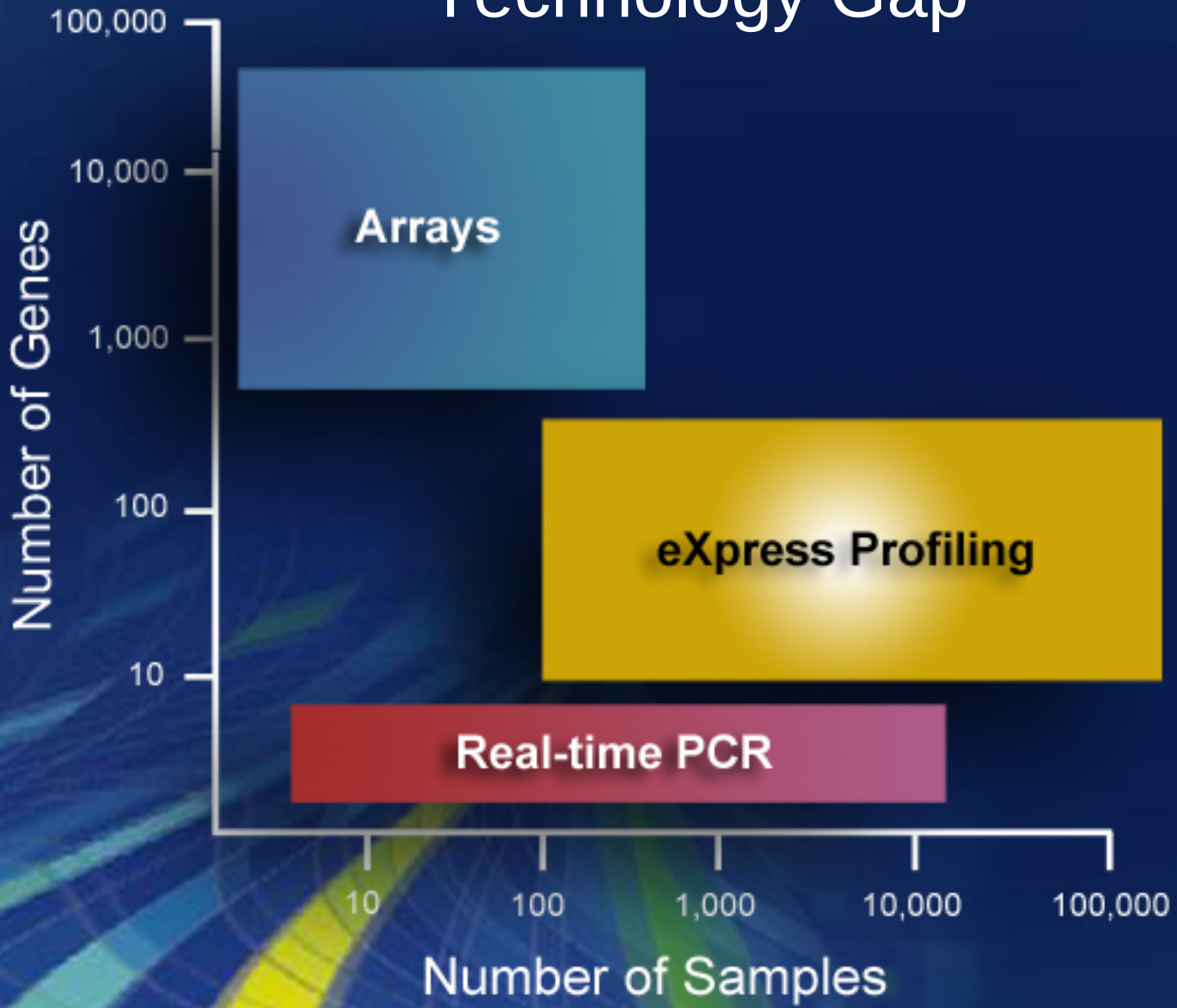
Assuring the Absence of Unknown or "Unsuspected" Agents

"However, non-specific molecular assays that can be used to low levels of occult, non-retrovirus RNA viruses, DNA viruses, or unusual agents are not available."

In evaluating viruses which may be latent or resident within cells… "This may require the development of non-specific (generic) assays to detect sequences of viruses in these families."

A Defined Risk Approach to the Regulatory Assessment of The Use of Neoplastic Cells as Substrates for Viral Vaccine Manufacture, U.S. FDA, CBER (OVRR) 17 March 2001 (pg.8 and 10)
Andrew Lewis, Phillip Krause and Keith Peden

New Tools Filling the Expression Technology Gap



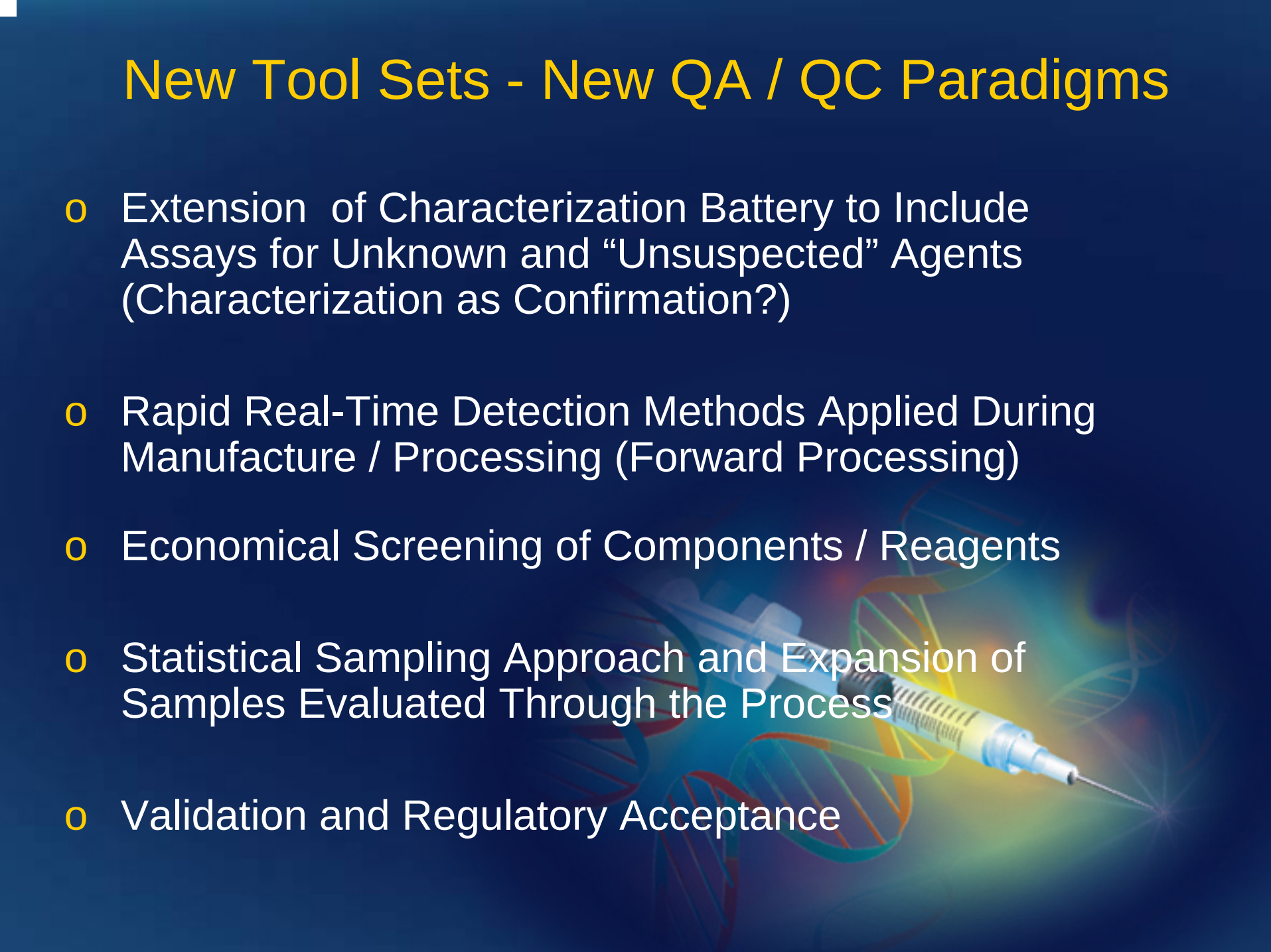
Features of eXpress Profiling

- Multiplexed low cost process
  - Solution-phase RT-PCR
  - High sensitivity (1 to 3 copies/cell/10^4 cells)
  - Low sample requirements (5 ng total RNA/reaction)
- Patented priming strategy
  - Maximizes dynamic range
  - Maintains relative gene ratio
- Off-the-shelf equipment and reagents
- Fluorescent capillary readout
- High-throughput capability

  PCR run as 20-30 plexes, multiple pooling strategies

New Tool Sets - New QA / QC Paradigms

- Extension of Characterization Battery to Include Assays for Unknown and “Unsuspected” Agents (Characterization as Confirmation?)
- Rapid Real-Time Detection Methods Applied During Manufacture / Processing (Forward Processing)
- Economical Screening of Components / Reagents
- Statistical Sampling Approach and Expansion of Samples Evaluated Through the Process
- Validation and Regulatory Acceptance



Case Study – QPCR for MVM

Contamination Events Led to the Development of Real time QPCR Assays for Detection of MVM:

- Assay Applied as a Screen for “Harvestability”*
- QC Test for Reagents / Barrier to Contamination*
- Investigation into the Sources of Potential Contamination*
- Viral Clearance Studies of Filters & Chromatography**

* Garnick, R.L., Dev Biol Stand. 1998;93:21-9.

** Zhan, D. et al, Biologicals Volume 30, Issue 4 , December 2002, Pages 259-270

Automated Sterility Testing System for Cell Therapy Products

Gary C. du Moulin, Ph.D.
Genzyme Biosurgery
Cambridge, MA 02139

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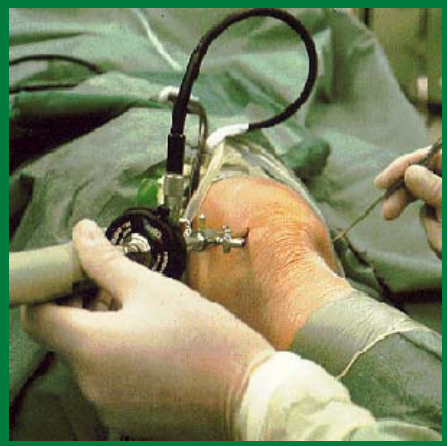
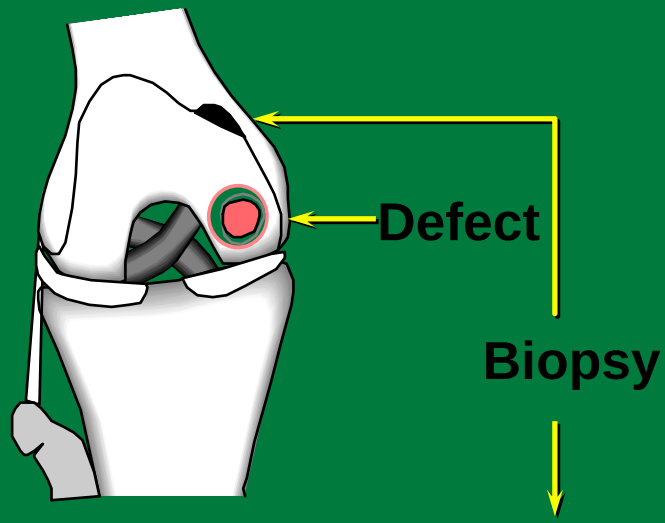
Autologous Cultured Chondrocyte Manufacturing Process

Indication: Repair injury to articular cartilage

Step 1 - Harvesting Biopsy





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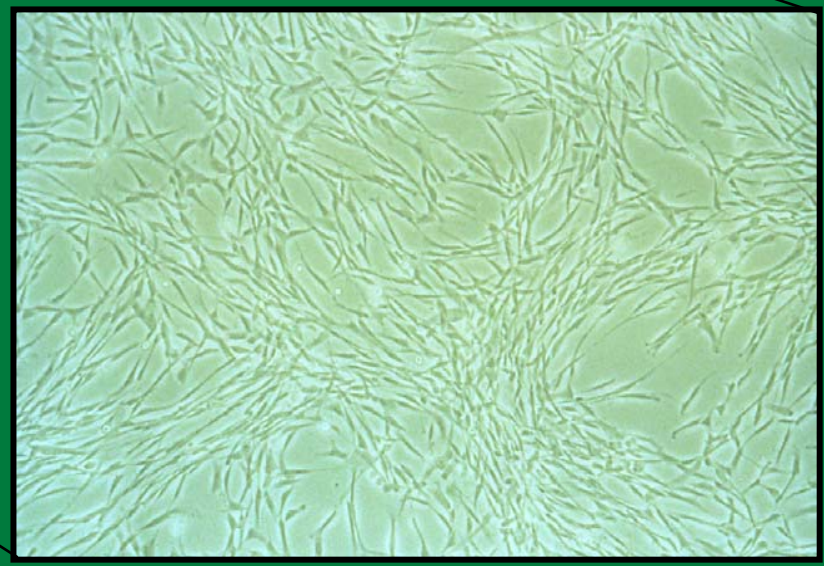
Step 2 - Biopsy Processing and Cell Culturing is performed in a Class 10,000 clean room



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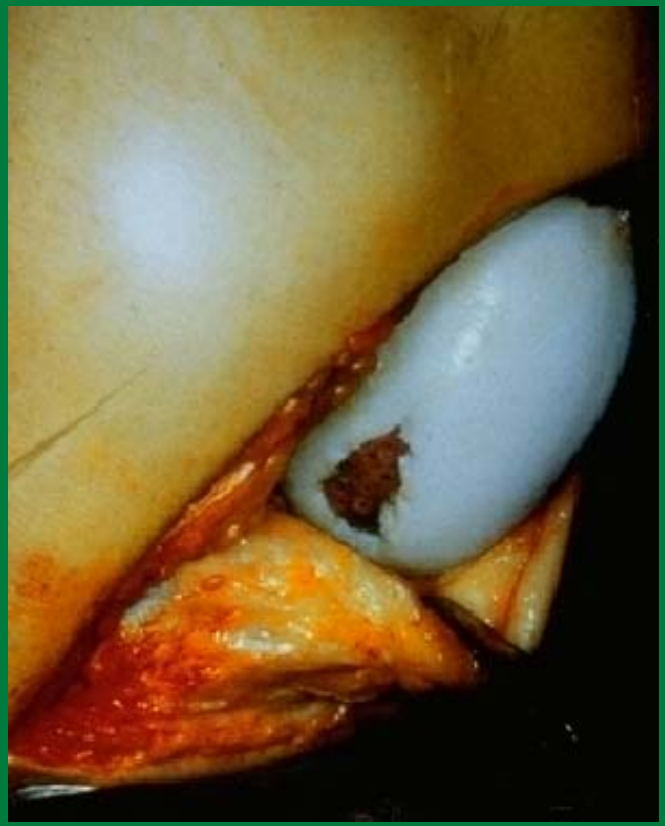
Autologous Cultured Chondrocyte Implantation



Prior to Implantation

Post Implantation

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Challenges in sterility testing for cell therapy and tissue engineered products

- Products have short shelf lives (24-48 hrs)
  - Compendial sterility testing takes 14 days to complete
- Cell suspensions difficult to interpret
  - Cells in media visually turbid and may generate false positive results
- 100% testing of autologous lots
  - Multiple testing points for each lot adds significant cost and product manipulation
- Non-continuous test sample readings
  - Loss of valuable time in notification

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Compendial Sterility Testing: A Challenge for Cell Therapy Products

- Short Shelf-life Products
  - 14 day incubation period exceeds product shelf life
    - Carticel® has a shelf-life of 3 days
    - Pre-Release strategy for lot release

Sample No.	Culture Phase	Sample Type	Status of Sterility Test at Product Release
1	Primary Phase	In-Process	Incubated for 14 days. Complete at final assembly
2	Expansion Phase	In-Process	Incubated for 14 days. Complete at final assembly
3	Product Phase	Pre-Release	Incubated for 4 days. **Incomplete** (10 days remaining)
4	Final Product Phase	Final Product	**Incomplete** (14 days remaining)

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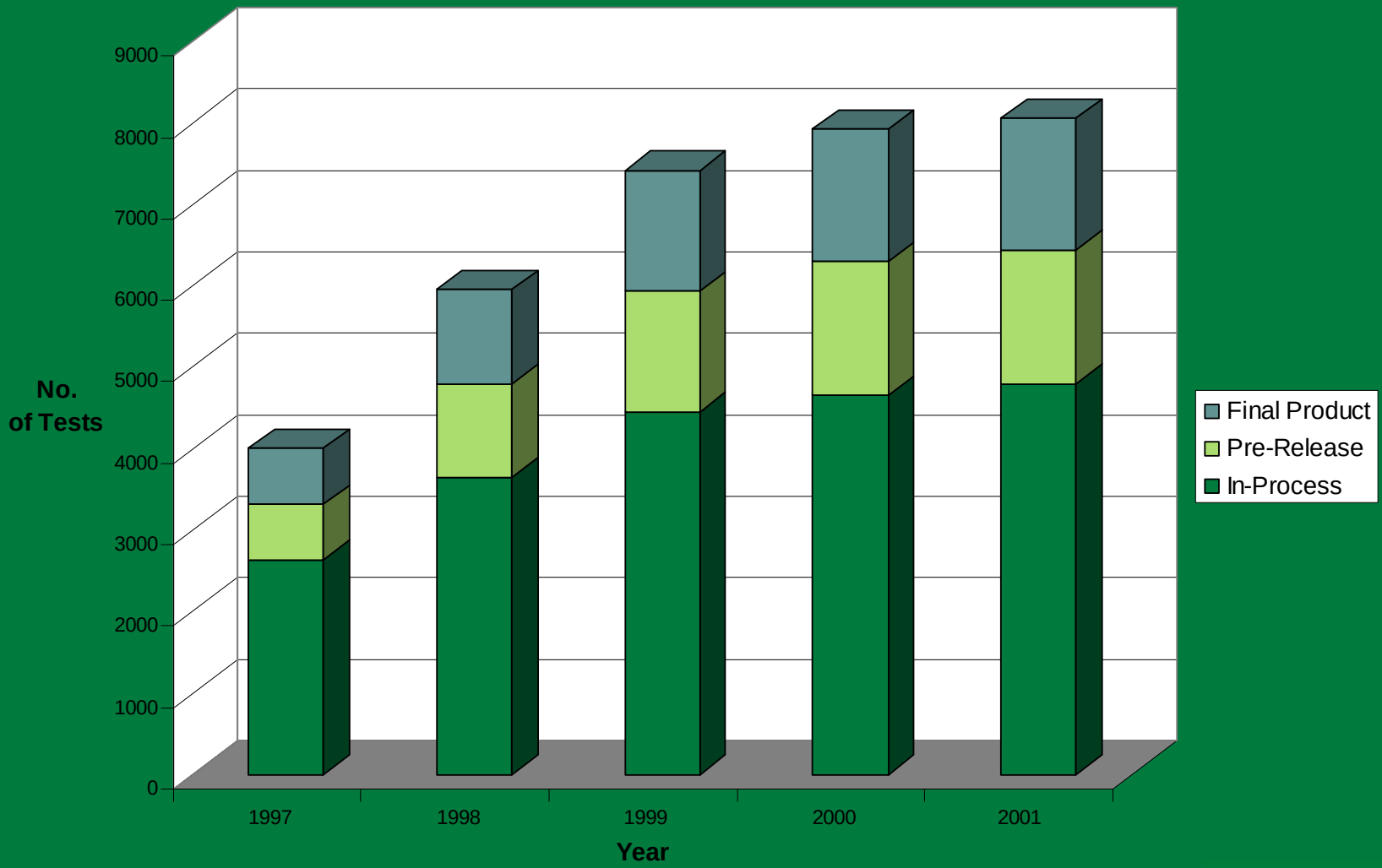
Carticel® Sterility Testing:



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Alternatives to Standard Test Methods

- Biological product regulations allow use of "equivalent methods and processes" (21 CFR 610.9) if they are equal to or greater than the assurances provided by the specified method
- Validate to show equivalency by end of Phase III
- Potential approach: perform "old" test concurrent with "new" rapid test to obtain data during product development

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Validation Phase I Overview

- Time commitment: 2 years
- Complexity
  - Installation Qualification
  - Operational Qualification
  - Bacteriostasis/Fungistasis
  - Engineering reports
  - Performance Qualification
- Length of Submission: 2048 pages

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BacT/Alert System

Colorimetric detection platform

Technology has been available for >10 years

Reliability as a major blood culture testing system in wide use.

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BacT/Alert System Colorimetric Technology

- CO_2 Sensor
- Silicon membrane freely permeable to CO_2
- Growing organisms produce CO_2 which diffuses across the membrane
- Free hydrogen ions interact with the sensor resulting in decrease in pH
- Sensor changes from green to yellow

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Specificity: *Ability to detect a range of Micro-organisms*

- *Staphylococcus capitis*
- *Staphylococcus warneri*
- *Staphylococcus epidermidis*
- *Pseudomonas aeruginosa*
- *Streptococcus pyogenes*
- *Candida parapsilosis*
- *Propionibacterium acnes*
- *Clostridium sporogenes*
- *Penicillium chrysogeum*
- *Aspergillus niger*

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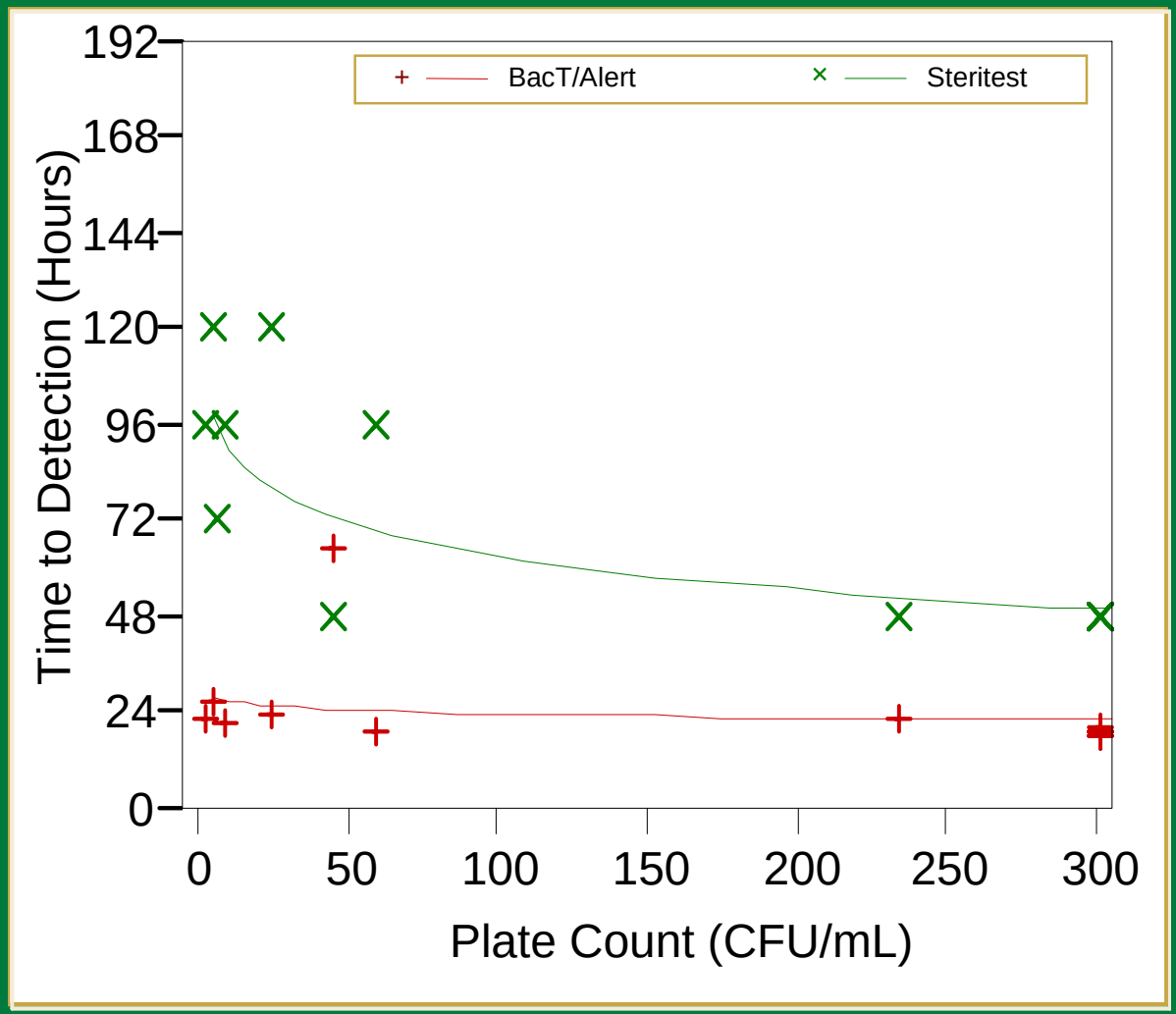
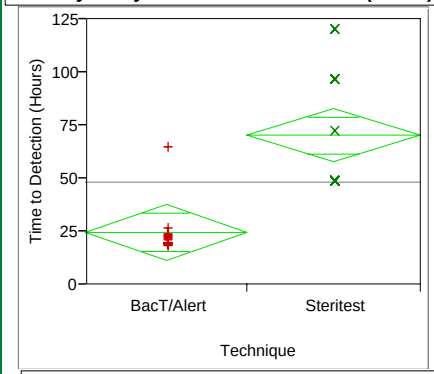
Limit of Detection: *Staphylococcus warneri*



Oneway Analysis of Time to Detection (Hours) By Technique



Oneway Anova

Summary of Fit

Rsquare	0.523893
Adj Rsquare	0.504849
Root Mean Square Error	22.58192
Mean of Response	48.32099
Observations (or Sum Wgts)	27

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	-45.619	-5.245	25	<.0001
Std Error	8.698			
Lower 95%	-63.532			
Upper 95%	-27.706			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Square	Mean Squar	F Ratio	Prob > F
Technique	1	14028.139	14028.1	27.5092	<.0001
Error	25	12748.577	509.9		
C. Total	26	26776.716			

Means for Oneway Anova

Level	Numbe	Mear	Std Erro	Lower 95%	Upper 95%
BacT/Alert	13	24.6667	6.2631	11.768	37.566
Steritest	14	70.2857	6.0353	57.856	82.716

Std Error uses a pooled estimate of error variance

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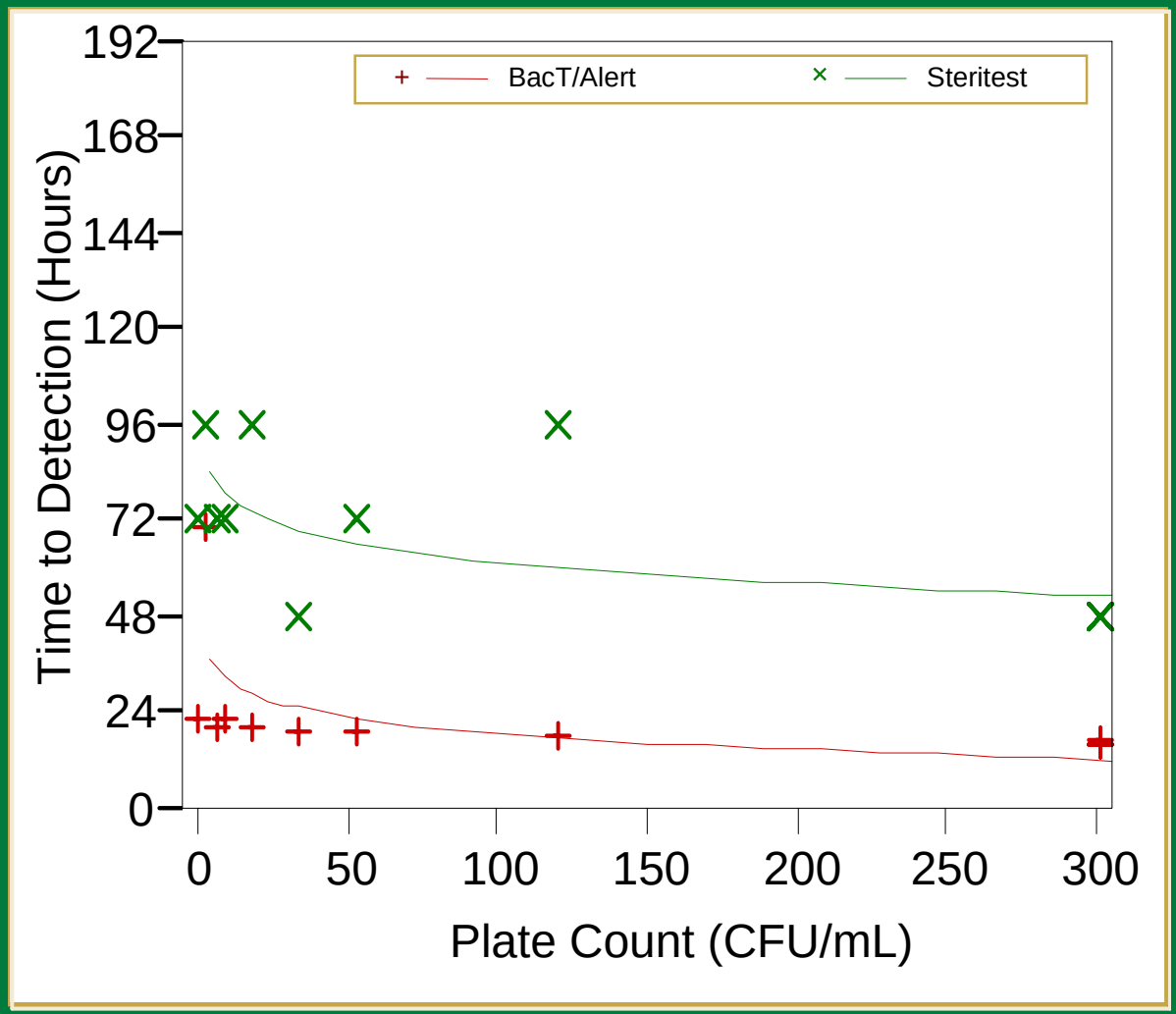
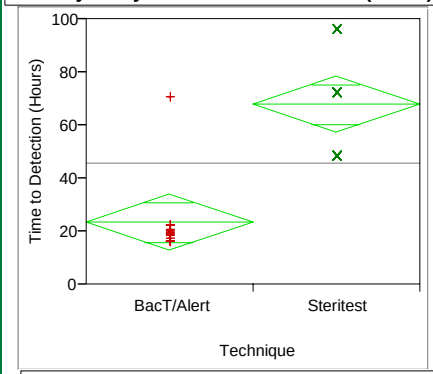
Limit of Detection: *Pseudomonas aeruginosa*



Oneway Analysis of Time to Detection (Hours) By Technique



Oneway Anova

Summary of Fit

Rsquare	0.632341
Adj Rsquare	0.615629
Root Mean Square Error	17.70759
Mean of Response	45.76597
Observations (or Sum Wgts)	24

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	-44.468	-6.151	22	<.0001
Std Error	7.229			
Lower 95%	-59.460			
Upper 95%	-29.476			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Square	Mean Squar	F Ratio	Prob > F
Technique	1	11864.448	11864.4	37.8380	<.0001
Error	22	6898.290	313.6		
C. Total	23	18762.738			

Means for Oneway Anova

Level	Numbe	Mear	Std Erro	Lower 95%	Upper 95%
BacT/Alert	12	23.5319	5.1117	12.931	34.133
Steritest	12	68.0000	5.1117	57.399	78.601

Std Error uses a pooled estimate of error variance

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Conclusion

- Patient specific Cell Therapy and Tissue Engineered products must be supported by quality control testing paradigms that are scalable and compatible with the product characteristics.
- Sterility testing must make use of existing and robust detection platforms that can conform to FDA requirements.
- Alternative testing methods must be validated for specificity, repeatability, limit of detection, ruggedness, and equivalence to the current method
- *We believe the BacT/Alert Microbial Detection System can meets the basic requirements to improve sterility testing of cell therapy products and be acceptable as an alternative testing method.*

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ALTHEA technologies, inc.

Thank you!